Mid-Infrared and Near-Infrared Spectral Properties of Mycorrhizal and Non-mycorrhizal Root Cultures

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We investigated the Fourier-transformed mid-infrared (MIR) and near-infrared (NIR) spectroscopic properties of mycorrhizal (M) and non-mycorrhizal (NM) carrot roots with the goal of finding infrared markers for colonization by arbuscular mycorrhizal (AM) fungi. The roots were cultured with or without the AM fungus *Glomus intraradices* under laboratory conditions. A total of 50 M and NM samples were produced after pooling subsamples. The roots were dried, ground, and scanned separately for the NIR and MIR analyses. The root samples were analyzed for fatty acid composition in order to confirm mycorrhizal infection and to determine the presence of fatty acid markers. Besides the roots, fatty acid standards, pure cultures of saprophytic fungi, and chitin were also scanned in order to identify spectral bands likely to be found in M samples. Principal components analysis (PCA) was used to illustrate spectral differences between the M and NM root samples. The NIR analysis achieved good resolution with the raw spectral data and no pretreatment was needed to obtain good resolution in the PCA analysis of the NIR data. Standard normal variate and detrending pretreatment improved the resolution between M and NM in the MIR range. The PCA loadings and/or the spectral subtraction of selected samples showed that M roots are characterized by absorbances at or close to 400 cm^{-1}, 1100–1170 cm^{-1}, 1690 cm^{-1}, 2928 cm^{-1}, and 5032 cm^{-1}. The NM samples had characteristic absorbances at or near 1734 cm^{-1}, 3500 cm^{-1}, 4000 cm^{-1}, 4389 cm^{-1}, and 4730 cm^{-1}. Some of the bands that differentiate M from NM roots are prominent in the spectra of pure fungal cultures, chitin, and fatty acids. Our results show that mycorrhizal and nonmycorrhizal root tissues can be differentiated via MIR and NIR spectra with the advantage that the same samples can then be used for other analyses.

Index Headings: **Fourier transform infrared spectroscopy; FT-IR spectroscopy; Near-infrared spectroscopy; NIR spectroscopy; Diffuse reflection; Mid-infrared spectroscopy; Principal component analysis; PCA; Fatty acids; Chitin; Fungi; Mycorrhizae.**

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi form symbiotic associations with the majority of row crops, frequently enhancing yields as well as soil quality.[1] Both the plant and the fungus benefit nutritionally in the AM symbiosis: The mycorrhizal fungus grows into the soil, improving nutrient uptake, and the fungus depends on the host plant for energy in the form of fixed carbon.[2,3]

Mycorrhizal fungi obtain carbohydrate from the host plant and store it as lipid.[3,4] Among these fungal lipids are unusual fatty acids such as 16:1ω5c and/or 18:1ω9c, which can be used to mark the presence and biomass of mycorrhizal fungi in environmental samples.[5–8]

Detection of mycorrhizal fungi by traditional methods has relied on microscopic observation and interpretation of morphological features.[9] Fatty acid analysis is an alternative method to staining and microscopy and may be faster, less prone to bias, and useful as a measure of biomass.[8] Fatty acid analysis, however, is not completely species specific[10] and requires destructive sampling, laborious wet chemistry procedures, and gas chromatography.

Near-infrared (NIR) and mid-infrared (MIR) diffuse reflectance spectroscopy have recently become established methods for the quick and reliable analysis of agricultural materials such as grains, forages, and soils.[11–14] The MIR range is advantageous because it has many distinct peaks amenable to spectral interpretation according to diagnostic bands,[15] while NIR spectra of complex materials consist of combination bands and overtones that may seem featureless without mathematical pretreatments.

Diffuse reflectance infrared spectroscopy is advantageous because ground powders can be analyzed directly without extraction or chemical treatments, and the sample is available for other analyses after the scans have been performed. Lipids have particularly strong absorbance in the infrared region. Recently, it has been demonstrated that NIR and MIR spectroscopy can be used for quantifying fatty acids (FA) in a variety of forage species.[14] MIR and NIR spectroscopy have been used for the classification of fungal isolates[16,17] as well as for differentiating plant material containing fungal growth from uninfected material.[18–20]

The objectives of this study were to (1) use multivariate statistics to compare the spectral properties of mycorrhizal and control roots in the NIR and MIR regions, (2) identify spectral regions that mark the presence of mycorrhizal fungi in the roots, (3) identify possible lipid or fungal spectral features that could be used to differentiate between mycorrhizal and non-mycor-

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FIG. 1. Micrograph of an axenic culture showing the transformed carrot roots, mycorrhizal hyphae, and chlamydospores. The picture was obtained through the eyepiece of a dissecting microscope.

rhizal root spectra, and (4) determine whether there is a relationship between root spectral data and fatty acid marker data.

EXPERIMENTAL

Carrot roots (*Daucus carota*) transformed by *Agrobacterium rhizogenes* Ri T-DNA were cultivated at 20 °C on a minimal nutrient medium,[21] modified to use Phytagel (Sigma) instead of agar. A relatively dilute Phytagel concentration of 2 g L^{-1} was used to facilitate the dissolution of the medium for an easier recovery of the roots and mycelium. To prepare the mycorrhizal (M) plates (Fig. 1), a 1 cm diameter plug containing roots, spores, and mycelium from a 4 month old culture of transformed carrot roots in symbiosis with *Glomus intraradices* (DAOM 181602) was placed in a fresh Petri dish with approximately 30 mL of medium. The non-mycorrhizal control (NM) cultures were prepared in the same way except that the fresh plates were inoculated with uninfected roots. The cultures were incubated for 12 weeks, which allowed for profuse root, mycelium, and spore production (Fig. 1). The cultures were examined with a dissecting microscope to confirm that the controls were free of fungal infection and to ensure that the inoculated roots had mycorrhizal infection. Eighty milligrams (80 mg) of sample material was needed to fill the diffuse reflection sampling cups and carry out the spectroscopic analysis. For this reason, duplicate culture plates were pooled in order to have enough material to fill the cups. Twenty-six NM and 24 M samples were analyzed.

Root Samples. At the end of the culture period, M and NM roots were removed from the Petri dishes and shaken gently with an orbital shaker in 100 mL 10-mmol sodium citrate, pH 6 at 37 °C to dissolve the Phytagel.[22] The samples were then rinsed with distilled water and retained with a 38 μm sieve to recover root and fungal material. The material was washed with water, frozen, lyophilized, and weighed. The M and NM roots were then ground with a mortar and pestle before spectroscopy and fatty acid analysis.

Fatty Acid Methyl Ester Analysis. Fatty acid analysis was carried out in order to indicate the presence and intensity of mycorrhizal infection in our samples, as well as to investigate the presence of possible fatty acid bands in the mycorrhizal spectra. Fatty acids were extracted from roots and material using the Microbial Identification System (Microbial ID, Inc. [MIDI], Newark, DE). The MIDI system is based on gas chromatography of saponified and esterified extracts followed by computerized peak identification.[23] The wet chemistry preparation of the samples consist of four steps: (1) saponification of fatty acids in 20 mg of freeze-dried fine roots with 2 mL 3.75 M NaOH in (methanol:water, 1:1 v:v) under heat (100 °C) for 30 min; (2) methylation of fatty acids by adding 4 mL of 6 M HCl in aqueous methanol:water (1:0.85 v:v) under heat (80 °C) for 10 minutes; (3) extraction of the fatty acid methyl esters (FAME) with 2 mL of 1:1 hexane:methyl-tert butyl-ether solution and rotating the samples end-over-end for 10 min; and (4) washing of the organic phases with 1.2% diluted NaOH by rotating the tubes end-over-end for 5 min. The organic phase (top phase) was analyzed in a 6890 GC Series II (Hewlett Packard, Wilmington, DE) equipped with a flame ionization detector and 25 m × 0.2 mm fused silica capillary column using ultra high purity hydrogen as the carrier gas. The temperature program was ramped from 170 °C to 250 °C at 5 °C min^{-1}. Fatty acids were identified and their relative peak areas were determined with respect to the other fatty acids in a sample using the MIS Aerobe method of the MIDI system. The FAME nomenclature is based on the number of C atoms, followed by a colon, the number of double bonds, and then by the position of the first double bond from the methyl (ω) end of molecules, *cis* isomers are indicated by "c", *trans* isomers are indicated by a "t", and branched fatty acids are indicated by the prefixes "I" and "a" for iso and anteiso, respectively. Other notations are "Me" for methyl, "OH" for hydroxyl, and "cy" for cyclopropane.

Infrared Spectroscopy. All M and NM root samples were scanned in the NIR and MIR on a Digilab FTS 7000 (Varian, Inc., Palo Alto, CA) Fourier transform spectrometer with a lead selenide detector and a quartz beam splitter for the NIR region or a deuterated triglycine sulfate detector and KBr beam splitter for the MIR range. A Pike AutoDIFF auto-sampler (Pike Technologies, Madison, WI) was used to obtain spectra in diffuse reflectance mode. Sulfur and KBr were used as background samples for the NIR and MIR, respectively. Spectral data were collected at 4 cm^{-1} resolution (64 co-added scans per spectrum) from 10 000 cm^{-1} to 4000 cm^{-1} for the NIR, and 4000 to 400 cm^{-1} for the MIR.

To determine lipid spectral features in the MIR and NIR, we scanned authentic samples of free fatty acids as well as commercially available oils. Free fatty acid samples of palmitic acid (16:0), palmitoleic acid (16:1), and oleic acid (18:1) (Sigma-Aldrich, St. Louis, MO) and pure canola and safflower oil were obtained for analysis. The fatty acids and oil samples were mixed with sulfur or KBr at a 5 percent by weight ratio and scanned in the NIR or MIR.

We obtained spectra from four different fungal cultures (*Phoma* sp., *Fusarium avenaceum*, *Fusarium equiseti*, and *Bipolaris sorokiniana*) in order to identify fungal bands that may also be present in the mycorrhizal roots. These four non-mycorrhizal fungal species were grown in pure culture in the laboratory. All are soil inhabiting fungi with the capability to infect plant tissue. The fungi were cultured at 20 °C in Petri plates of potato dextrose broth solidified with Phytagel,

TABLE I. **Fatty acid data as percent of total fatty acids. Only fatty acids with more than 12 carbons in the aliphatic end of the molecule and making more than 0.5 percent of total peak area are included. The TTEST results are the probability that the mycorrhizal (M) and non-mycorrhizal (NM) data come from populations with the same mean according to a Student's t-Test. Summed feature 7 contains 18:1ω7c, 18:1ω9t, and/or 18:1ω12t. Summed feature 5 (18:0anteiso/18:2ω6,9c). n = 26 non-mycorrhizal, and 24 mycorrhizal.**

	NM	M	TTEST
12:0	0.99	0.64	0.00
13:0anteiso	0.43	0.27	0.00
15:0	0.60	0.53	0.07
16:1iso	0.36	0.15	0.00
16:1ω5c	0.04	14.18	0.00
16:0	25.19	26.92	0.30
16:02OH	1.03	0.89	0.02
18:1ω9c	6.83	6.46	0.48
18:1ω7c	0	1.61	0.00
18:0	4.04	3.67	0.13
19:0iso	0.32	0.30	0.55
20:0	4.29	3.89	0.12
Summed feature 5	38.4	26.63	0.00
Summed feature 7	10.57	7.52	0.00

allowing for the dissolution of the medium and separation of the fungal biomass at the end of the growth period (as with the root cultures above). The fungal cultures were ground in liquid nitrogen using a mortar and pestle before being scanned undiluted in the MIR and NIR as with the root samples.

Chitin (poly-N-acetylglucosamine) (Sigma-Aldrich, St. Louis, MO) was ground with an agate mortar and pestle and scanned in the MIR and NIR as with the root and fungal samples, except that a liquid nitrogen cooled indium antimonide detector was used for the NIR.

Multivariate Analysis and Spectral Subtraction. Principal component analysis (PCA) of the MIR and NIR spectra, as well as the correlation of spectra and constituents were carried out using GRAMS/AI Version 7.02 (Thermo Galactic, Salem. NH). The whole wavenumber regions in the MIR and NIR were used for the PCA analyses except for two small regions in the MIR between 2300–2385 cm^{-1} and 660–665 cm^{-1}, which were affected by small fluctuations possibly due to CO_2. Removing these regions did not affect the PCA results but excluded the possible CO_2 bands from the component loadings analysis. GRAMS/AI was used to obtain the average and subtracted spectra of selected samples. The standard normal variate (SNV) and detrending pretreatment of GRAMS/AI was used for the PCA of the MIR range. The SNV and detrending are commonly used in NIR spectroscopy to reduce baseline shift, tilt, and curvature due to sample heterogeneity and scattering issues during diffuse reflection scans. The SNV treatment improved the resolution of the M and NM samples in the MIR, but not the NIR, which had good resolution with the untreated (raw) spectral data. PCA loadings were used to indicate the strength of the correlation between components and the wavenumbers. For spectral subtraction, the spectra were standardized by subtracting each individual wavenumber from the mean absorbance of the spectrum, then dividing by the standard deviation of the spectrum.

RESULTS AND DISCUSSION

Few studies on the spectroscopy of infected plant tissues have been carried out to date. However, infrared spectroscopic

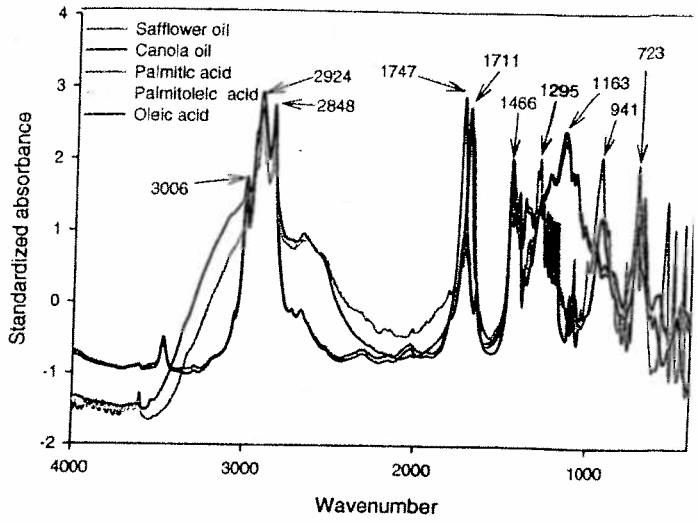

FIG. 2. Mid-infrared spectra of free fatty acids and oils.

techniques can be used successfully to distinguish uninfected plant material from samples infected with fungi.[24,25] Gordon et al. used transient infrared spectroscopy to acquire infrared spectra from a moving bed of corn kernels and were able to distinguish good corn from grain infected with *A. flavus*.[24] Eukhimovitch et al. used infrared microscopy to reliably identify potato samples infected with *C. coccodes*.[25] These results suggest that infrared spectroscopic methods can be developed to diagnose fungal infection for many mycological applications in a quick, inexpensive, and sensitive fashion. Mycorrhizal research could benefit greatly from such a technology because quick screening of field samples will allow for frequent, detailed, and more meaningful data from relatively large field experiments. This would require much more manpower if the traditional root clearing, staining, and visual scoring approach were used. The recent use of fatty acid marker analysis, a fast and unbiased technique based on wet chemistry and gas chromatography, needs to be used with caution because species like *G. occultum*, an ubiquitous mycorrhizal fungus, does not have the 16: 1ω5 marker.[5] A possible approach could be to use whole spectra together with multivariate statistical techniques in order to differentiate the samples. Alternatively, specific diagnostic wavelengths, once identified, could be used in order to mark the presence of fungal material in a plant sample.

Fatty Acid Analysis of Roots. The AM fungi contain unusual fatty acids that are not present, or are present only in smaller amounts, in uninfected roots or rhizosphere microorganisms.[26] Because of this, M roots can be distinguished from NM roots by their fatty acid profiles.[5] For example, 16:1ω5c, 18:1ω7c, 20:1ω9c, 20:2ω6c, 20:3, 20:4, and 20:5 occur solely or in significantly higher concentrations in M roots relative to uninfected roots.[5,7] There is no universal fatty acid marker for all mycorrhizal fungal species, but the aforementioned fatty acids alone or in combination have been proposed as markers of different mycorrhizal fungi in environmental samples and roots.[5,6] Root cultures have the advantage over environmental samples in that there is no background of fatty acids pertaining to other rhizosphere organisms or soil. In this study, M carrot roots had a significantly higher percentage of 16:1ω5c and 18:1ω7c than NM roots (Table I). The percentage of 16:1ω5c within the M samples varied from 42% to below detection

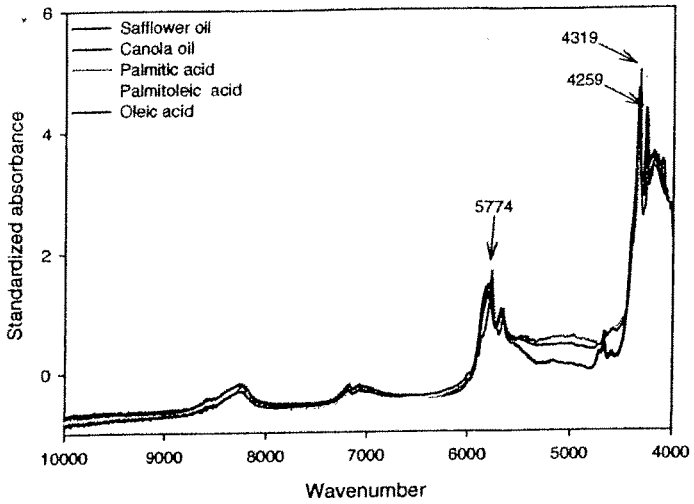

FIG. 3. Near-infrared spectra of free fatty acids and oils.

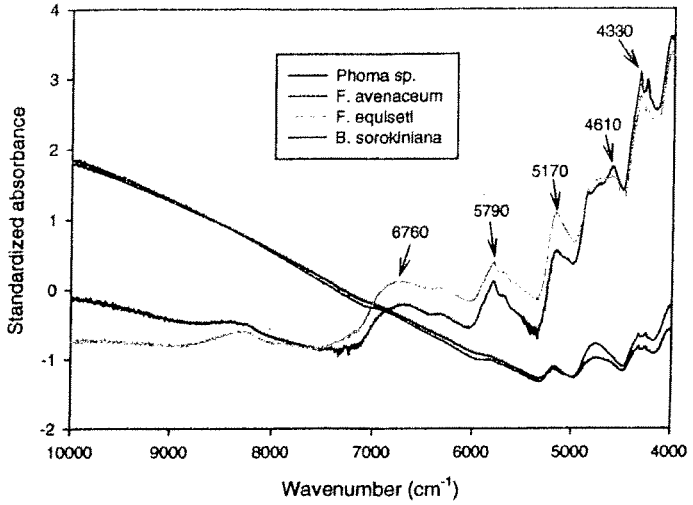

FIG. 5. Near-infrared spectra of fungal pure cultures.

limits, suggesting that the M samples had wide variation in the amount of mycorrhizal infection. The relative percentage of fatty acids 15:0, 16:0, 18:0, 18:1ω9c, 19:0iso, and 20:0 were statistically indistinguishable between M and NM roots ($p > 0.05$). The NM roots had significantly higher percentage of 12:0, 13:0anteiso, 16:1iso, 16:02OH, and two unresolved mixtures of fatty acids (18:1ω7c, 18:1ω9t, and/or 18:1ω12t, 18:0anteiso, and/or 18:2ω6,9c) than NM roots.

Spectral Properties of Free Fatty Acids and Oils. The MIR spectra of the fatty acids and oils show some common features (Fig. 2). All free fatty acids, as well as the canola and safflower oils, had peaks at 2924 cm^{-1}, 2848 cm^{-1}, 1466 cm^{-1}, and 723 cm^{-1}. The bands at 2924 cm^{-1} and 2848 cm^{-1} are caused by CH_2 stretching, absorbance at 1466 cm^{-1} is due to CH_2 and CH_3 bending, and absorbance at 723 cm^{-1} is caused by $(CH_2)_n$ and HC=CH bending.[27] The free fatty acids have higher absorbance than the oils at two broad regions spanning 2200–2800 cm^{-1} and 3030–3400 cm^{-1}. The monounsaturated fatty acids (palmitoleic and oleic acids) have nearly indistinguishable MIR spectra. The monounsaturated fatty acids had a peak at 3006 cm^{-1} not found on the saturated palmitic acid, due to stretching of the $=CH_{cis}$ double bond. [27] Palmitic acid has pronounced peaks at 723 cm^{-1}, 941 cm^{-1}, and 1295 cm^{-1} that were less marked on the rest of the samples. The free fatty acids had a prominent peak at 1711 cm^{-1} attributable to –C=O stretching in the carboxylic end of the molecule, while the oils had an adjacent peak at 1747 cm^{-1} due to –C=O in esters. The oils have a peak at 1163 cm^{-1} (–C–O, or $-CH_2$ stretching and bending), which is absent in the free fatty acid spectra.

The NIR spectra of the free fatty acids and oils shows that all the spectra had peaks at 5774 cm^{-1}, 5683 cm^{-1}, 4319 cm^{-1}, and 4259 cm^{-1} (Fig. 3). The peaks at 5774 cm^{-1} and 5683 cm^{-1} are in the first overtone region for methylene stretching in linear aliphatic molecules. The peaks at 4319 cm^{-1} and 4259 cm^{-1} are in the first combination region for –CH stretching with the bending and stretching of the MIR region.

Spectral Properties of Fungal Material and Chitin. The MIR spectra of the pure fungal cultures shows that fungi have a broad peak at around 3400 cm^{-1}, peaks at 2928 cm^{-1}, 1666 cm^{-1}, 1554 cm^{-1}, 1157–1085 cm^{-1}, and a broad peak around 580 cm^{-1} (Fig. 4). The peaks near 1085 cm^{-1} and 1554 cm^{-1}, as well as a peak near 1405 cm^{-1}, have been identified as useful in the detection of fungal infection in potato tissue.[25]

The NIR scans show that the pure fungal cultures have

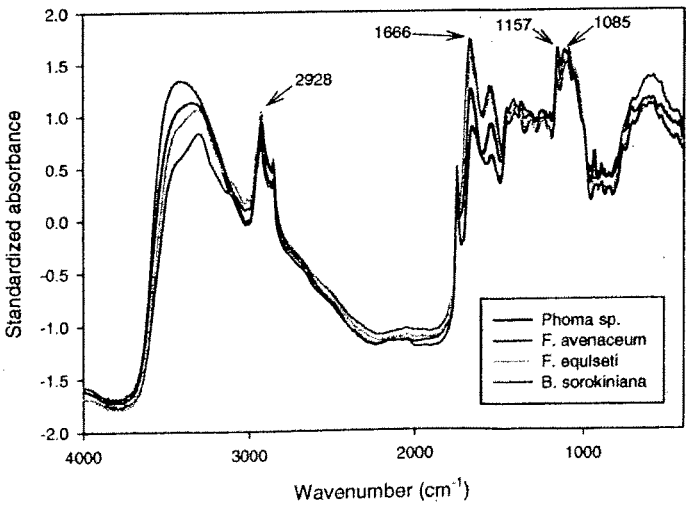

FIG. 4. Mid-infrared spectra of fungal pure cultures.

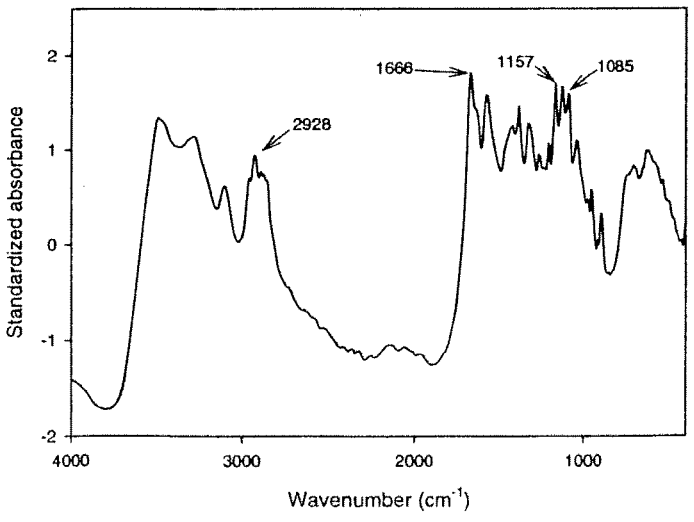

FIG. 6. Mid-infrared spectrum of chitin.

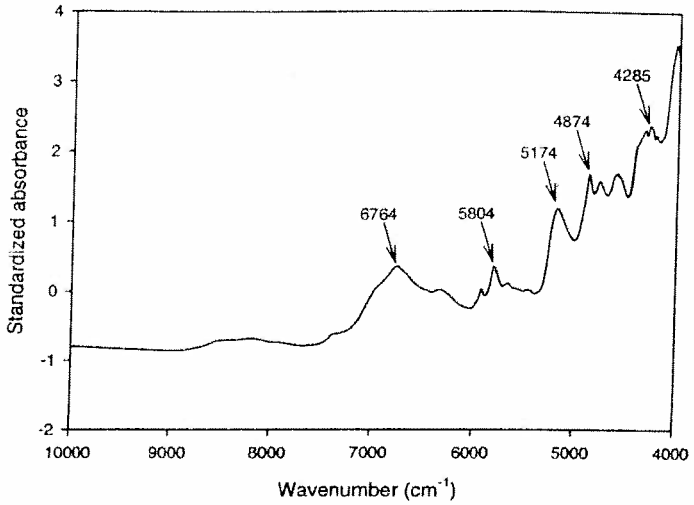

FIG. 7. Near-infrared spectrum of chitin.

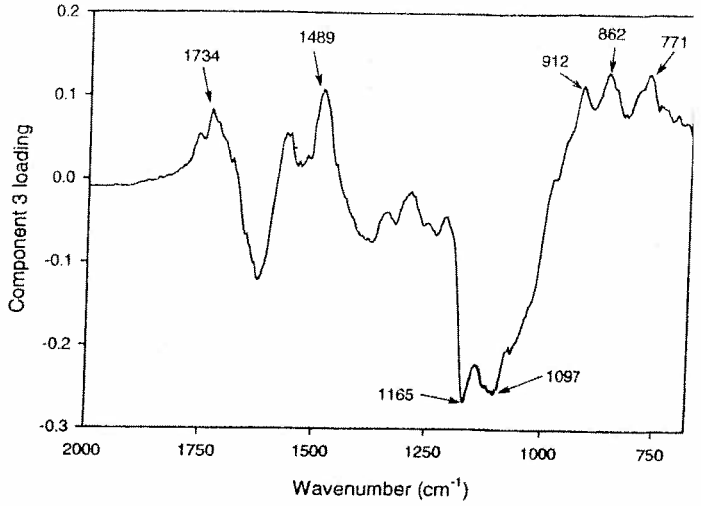

FIG. 9. Component 3 loadings for the PCA of the raw mid-infrared spectra of the mycorrhizal and non-mycorrhizal axenic cultures. The spectral range with the highest loadings is shown.

different spectral features. The *Phoma* sp. and *Bipolaris sorokiniana* spectra have high absorbance towards wavenumbers higher than 7000 cm^{-1}, possibly because of their darker color (Fig. 5). However, the NIR spectra of the other fungal material had features at 5790 cm^{-1}, 5675 cm^{-1}, 5170 cm^{-1}, 4610 cm^{-1}, 4330 cm^{-1}, 4260 cm^{-1}, and 4020 cm^{-1}, although these peaks were not as pronounced as those shown in the MIR (Fig. 4).

The MIR and NIR spectra of chitin (Figs. 6 and 7) show many similarities with those of the pure fungal cultures. This suggests that chitin, besides being an important fungal cell wall component, contributes significantly to the absorbance in the MIR and NIR of the fungal material. The chitin and the fungal spectra share prominent peaks at 1085 cm^{-1}, 1157 cm^{-1}, 1666 cm^{-1}, and the close peaks at 2928 cm^{-1} and 2924 cm^{-1} in the MIR, and at 4020 cm^{-1} and close bands at 5170 cm^{-1} and 5174 cm^{-1} (Figs. 4 through 7).

Principal Component Analysis of the Root Mid-Infrared Spectra. The PCA analysis of the MIR shows that M roots tend to have different MIR spectral properties than NM roots, although the difference was not clear cut (data not shown). The

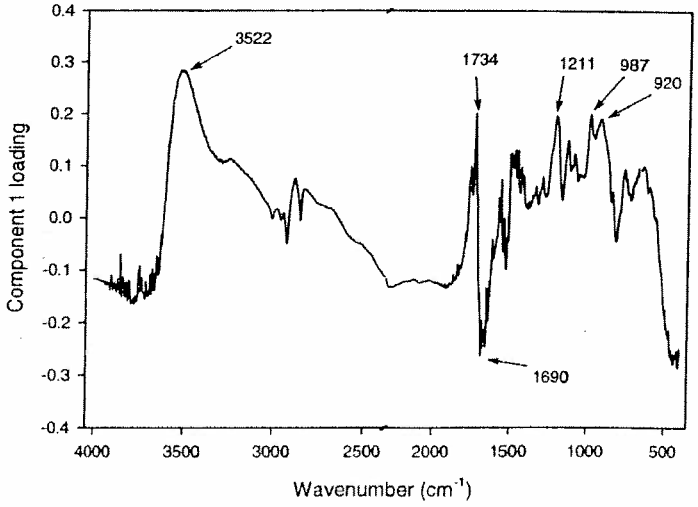

FIG. 8. Component 1 loadings for the PCA of the raw mid-infrared spectra of the mycorrhizal and non-mycorrhizal axenic cultures.

PCA can help concentrate the large amount of information included in the spectra into a few underlying components. The scores indicate the location of the sample along a component, and samples with close scores tend to have similar spectral properties. Mycorrhizal spectra tend to have low scores along components 1 and 3 of the PCA compared to the NM spectra. The relationship between the sample scores and the spectral data is determined by the factor loadings (Figs. 8 and 9). The sample score distribution along the axes is positively related to wavelengths with high loadings and negatively related to wavelengths with low loadings for the particular axis. Factor loadings for component 1 indicate that absorbances at several close peaks near 1690 cm^{-1} are positively related to the presence of mycorrhizae, while absorbances at a broad band around 3522 cm^{-1} and peaks at 1734 cm^{-1}, 1211 cm^{-1}, and 987–920 cm^{-1} are associated with non-mycorrhizal roots (Fig. 8). The loadings for component 3 of the PCA, which accounts for 12.4% of the variation in the spectral data, indicate that M samples are associated with wavenumbers between 1097 and 1165 cm^{-1} (Fig. 9). The high scores along component 3, where the NM samples tend to be, are associated with loading peaks at 1734 cm^{-1}, 1489 cm^{-1}, and several peaks between 771 and 912 cm^{-1}.

As discussed above, the SNV and detrending pretreatments improved the resolution of M and NM samples. One possible reason for the lack of clear separation with the raw MIR spectra is that some of the differences of importance between M and NM samples are in the area around 1100 cm^{-1}, where specular reflection distortions are prevalent in the MIR. Diluting the samples with KBR is known to reduce the specular reflection issues, but it adds significant labor to the analysis. The mathematical pretreatment, however, is instantaneous and improves the resolution of the M and NM samples satisfactorily.

Principal Component Analysis of the Root Near-Infrared Spectra. The principal component analysis of the raw NIR spectral data shows that the M roots have distinctly different NIR spectra than the NM roots (data not shown), and no pretreatment was needed to achieve good resolution between NM and M roots in the NIR region. Mycorrhizal roots have lower scores than NM roots for components 2 and 3,

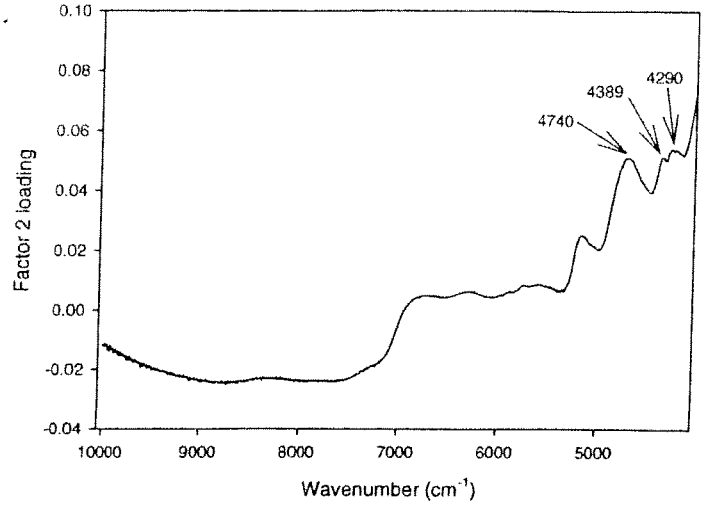

FIG. 10. Factor 2 loadings for the PCA of the raw near-infrared spectra of the mycorrhizal and non-mycorrhizal axenic cultures.

which explain a small portion of the spectral variation (1.6 percent altogether). The loadings pattern for component 2 looks similar to a raw root NIR spectrum, with the smaller wavelengths (near 4000 cm^{-1}) explaining the score patterns of NM samples, as well as broad regions near 4740, 4389, and 4290 cm^{-1} (Fig. 10). Loadings for component 3 indicate that NM roots have high absorbance at wavenumbers above 9100 cm^{-1} and at 4730 cm^{-1}, 4276 cm^{-1}, and 4000 cm^{-1}, while M roots are associated with absorbance at 5032 cm^{-1} and broad areas between 7100–7570 cm^{-1} and 5300–5370 cm^{-1} (Fig. 11).

Average Spectra and Spectral Subtraction. The subtracted MIR spectrum of selected samples shows the different spectral properties of M and NM tissues (Fig. 12). The subtracted spectra illustrate the difference in spectral properties between highly mycorrhizal roots and uninfected controls. This goes beyond the PCA approach, which shows the differences between the whole set of inoculated cultures and controls, regardless of the intensity of mycorrhizal infection. The M samples were selected to have high 16:1ω5 percentage, indicating high infection. The subtracted spectrum shows the

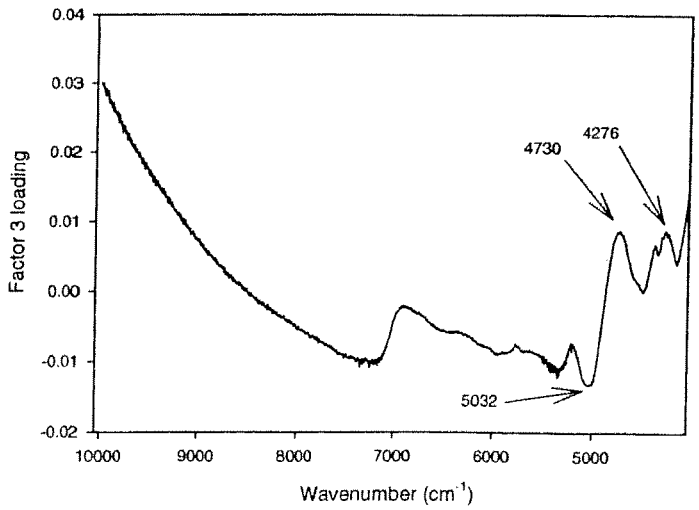

FIG. 11. Factor 3 loadings for the PCA of the raw near-infrared spectra of the mycorrhizal and non-mycorrhizal axenic cultures.

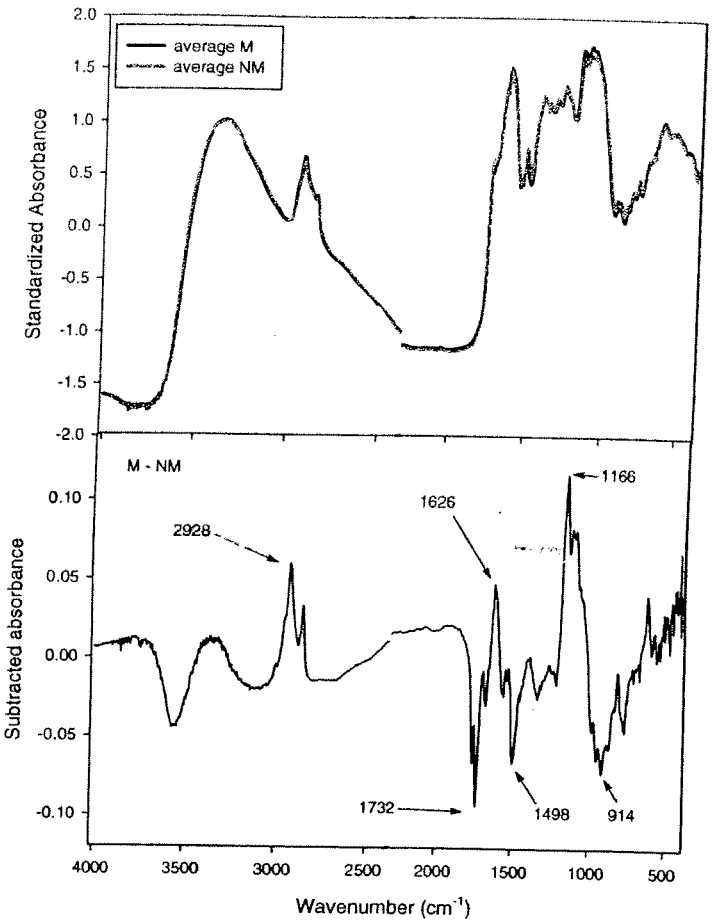

FIG. 12. **(Top)** Average mid-infrared root spectra of selected mycorrhizal and non-mycorrhizal axenic samples, and **(bottom)** the subtracted (non-mycorrhizal − mycorrhizal) spectrum. $n = 3$.

different spectral properties of M and NM samples. The selected mycorrhizal roots were most different from NM roots near 400 cm^{-1}, at 1626 cm^{-1}, 2928 cm^{-1}, and a region between 1150–1180 cm^{-1} that peaked at 1166 cm^{-1}. Non-mycorrhizal roots were characterized by absorbance at a broad region around 3670 cm^{-1}, at 1732 cm^{-1}, 1498 cm^{-1}, and 914 cm^{-1}. Note that bands near 1165 cm^{-1}, 1734 cm^{-1}, 912 cm^{-1}, 1490 cm^{-1}, and 3600 cm^{-1} were also important in resolving M and NM samples using the PCA analysis (above).

The MIR spectra are amenable to interpretation because they have distinct peaks that can be attributed to specific spectral bands.[15] While the chemistry surrounding the functional groups can cause shifts on the specific bands, the PCA and subtraction approaches can indicate likely differences in composition between M and NM roots. The 1732 cm^{-1} band has been attributed to C=O stretching[28] and suggests a higher amount of esterified carbohydrates in the NM samples than in the infected roots. The NM roots are also characterized by absorbances near 1490 cm^{-1} and 914 cm^{-1}, possibly due to C=C stretching within aromatic hydrocarbons, and =CH or =CH_2 bending, respectively. The PCA and the subtraction approach show that the M samples are characterized by absorbances at 1100–1170 cm^{-1}, which could be attributed to –C–C–C or –O–C bond stretching. The absorbance at 2928 cm^{-1} shown by the subtraction approach suggests increased –CH, –CH_2, or CH_3 stretching in the M samples. The band at

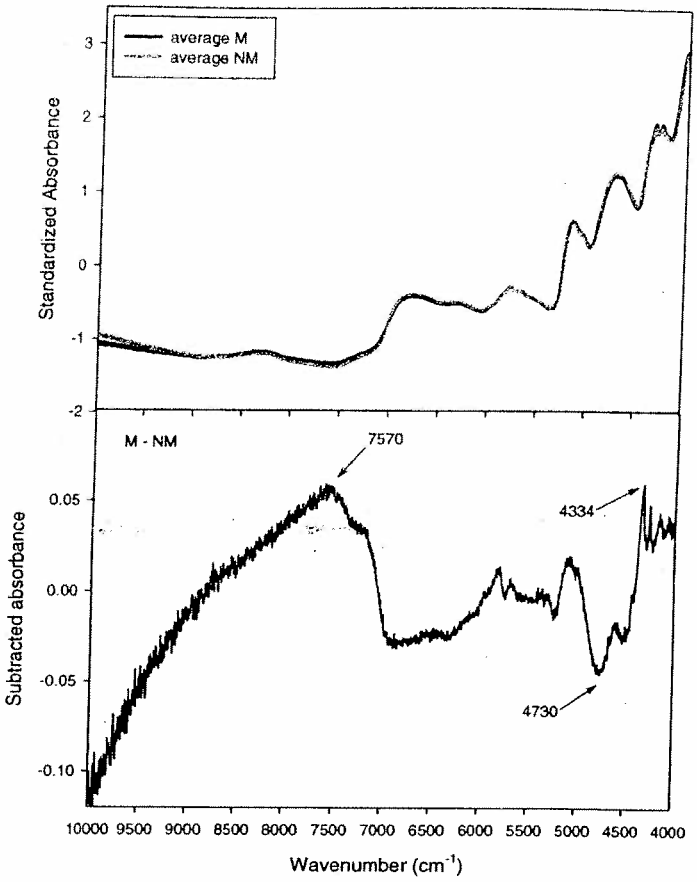

FIG. 13. (**Top**) Average near-infrared root spectra of selected mycorrhizal and non-mycorrhizal axenic samples, and (**bottom**) the subtracted (non-mycorrhizal − mycorrhizal) spectrum. $n = 3$.

1626 cm^{-1} in the M samples is near the C=C stretching band for alkenes.

The NIR spectra of three M samples (the same ones shown in Fig. 12) were averaged and subtracted from the NM spectra in order to illustrate the spectral differences between highly infected and control roots (Fig. 13). The M samples were different from NM samples at a broad section around 7570 cm^{-1} and higher wavenumbers and at a small peak at 4334 cm^{-1}. The NM NIR spectra were different than the M at a broad peak around 4730 cm^{-1} and at 10 000 cm^{-1}. Note that these wavenumbers were also important in the component loadings to differentiate M and NM roots in the PCA analysis above.

Comparison of the Root Spectral Analyses and the Fungal, Fatty Acid, and Chitin Spectra. The MIR PCA and the spectral subtraction analyses show that the M roots are distinguished from the NM roots by higher absorbance at wavenumbers close to 1165 cm^{-1} (Figs. 9 and 12). This is a prominent band in safflower and canola oil MIR spectra (Fig. 2) and is close to the peak at 1157 cm^{-1} observed in the pure fungal biomass (Fig. 4) and chitin (Fig. 6).

The subtraction of the NIR spectra of highly infected M roots and NM roots identifies 4334 cm^{-1} as one of the NIR absorbance features that differentiates M from NM spectra (Fig. 13). This band is close to peaks present in the *Fusarium* fungal biomass at 4330 cm^{-1} (Fig. 5) and at 4319 cm^{-1} in the fatty acids and oils (Fig. 3), which suggests that fungal and lipid components of the M roots differentiate them from NM roots.

CONCLUSION

This study demonstrates the applicability of alternate spectroscopic methods for the detection of M fungi in roots Ri T-DNA carrot roots colonized by *G. intraradices* have distinct spectra relative to NM roots in the NIR and MIR ranges. Replicate cultures of the mycorrhizal and controls clustered separately in the PCA diagrams of the raw NIR spectra and the pretreated MIR spectra, indicating good reproducibility of the spectroscopic technique. No pretreatment was needed to achieve good resolution of the PCA in the NIR, while standard normal variate pretreatment of the MIR data improved the separation of the M and NM samples. Our results show that mycorrhizal fungal components such as lipids and chitin can partially explain the spectral differences between M and NM roots. Future work should investigate batch effects and culture conditions on the spectral properties of M and NM roots. Also, the spectral properties of other crop–fungal species combinations in the laboratory and the field need to be determined in order to establish whether there are universal spectral signatures for this important group of organisms.

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